

**JP 63004895 : Japanese b-encephalitis vaccine**  
**, Saikin Seizai Yoka, 1963**

Japanese B-encephalitis vaccine prepared from infected mouse brain by centrifuging produces a reaction when injected subcutaneously into Hartley sensitised guinea pigs. The reaction arises from some substance present in the lipo-protein fraction and is eliminated by treatment of the centrifugate with protamine sulphate followed by adsorbents such as acid clay or charcoal.

Japanese B-encephalitis virus, Nakayama strain was inoculated into a mouse brain and 5 days later the mouse, showing signs of encephalitis was killed by bleeding. The brain was removed aseptically and homogenised with 9 vols. of physiological saline. The homogenate was centrifuged for 30 min. at 3000 r.p.m. to give a cloudy supernatant A. To this was added 1 mg/ml. of protamine sulphate and after shaking, the mixture was neutralised and allowed to stand for 2 hrs. at 4 deg., then centrifuged to give a reddish brown supernatant B. Acid clay (2% w/v) was added to B and after shaking, neutralising and allowing to stand for 4 hrs. at 4 deg. the mixture was again centrifuged to give a clear yellow supernatant C.